at least about 150 mM of a soluble salt,

an effective amount of a non-ionic detergent sufficient to release RNA from the biological sample without causing viscosity due to release of chromosomal DNA, and

a solid support to which is joined an immobilized oligonucleotide comprising a nucleotide base sequence [capable of forming] which forms a stable immobilized oligonucleotide:RNA hybridization complex;

c) separating the hybridization complex joined to the solid support from unhybridized sample components; and

d) then washing the hybridization complex joined to the solid support with a solution having sufficient salt concentration to maintain the hybridization complex.

REMARKS

Oath/Declaration

The Examiner stated that a new oath or declaration was required because the oath was not executed in accordance with 37 C.F. R. 1.66 or 1.68.

An executed declaration was mailed to the U.S. Patent and Trademark Office on October 20, 1999 with authorization to deduct the required fee from Assignee's deposit account. The executed declaration was received on October 25, 1999. Applicants believe this declaration is fully compliant with the rules.

Changes to the Specification

The changes to the specification regarding the priority claim as suggested by the Examiner have been made.

Amended Claims

Claims 1-20 are pending; all claims have been rejected. Claims 1-6, 9, 10, 16, 18 and 19 have been amended. No new matter has been added by these amendments. Support for the "isothermal" amendment to claims 1 and 9 is found at page 6, line 14 and by further reference to page 17, lines 20-26 and page 18, lines 7-13. Entry of this amendment and reconsideration of this application is respectfully

requested.

Rejections under 35 U.S.C. § 112, Second Paragraph

Claims 1-20 have been rejected under 35 U.S.C. § 112, second paragraph. The Examiner objected to the term "capable of" and "incapable of" as indefinite. Claims 1-6, 9, 10, 16, 18 and 19 have been amended by deleting the objected to terms and replacing the terms with appropriate definite language.

Based on these changes, Applicants respectfully request allowance under 35 U.S.C. § 112, second paragraph of Claims 1-20.

Rejections under 35 U.S.C. § 102(b)

Claims 19 and 20 have been rejected under 35 U.S.C. § 102(b) based on the disclosure of Saunders et al. (U.S. Pat. No. 5,066792).

For a reference to anticipate an invention there must be no difference between the claimed invention and the reference disclosure, as viewed by a person of ordinary skill in the field of the invention. *Scripps Clinic & Research Fndn. v. Genetech*, Inc., 927 F.2d 1565, 18 USPQ2d 1001 and 1896 (Fed. Cir. 1991).

Applicants respectfully argue that differences exist between the claimed invention and the RNA extraction method taught by Sauders et al. First, Applicants specify use of "a non-ionic detergent" in the solution mixed with the biological sample. In contrast, Saunders et al. (col. 5, lines 34-36) used Sarcosyl (i.e., N-lauroylsarcosine, usually supplied as a sodium salt), is an anionic detergent, as shown by the attached **Exhibit 1** (SIGMA 1999 Catalog of Biochemicals and Reagents for Life Science Research, pp. 626, 933 and 1543). N-lauroylsarcosine (also known as sodium lauryl sarcosinate) is used to lyse cells, for example, as shown in the protocol of Sambrook et al., Molecular Cloning, A Laboratory Manual, 2nd Edition (Cold Spring Harbor Laboratory Press, 1989, Cold Spring Harbor, NY) at page 7.20 (attached **Exhibit 2**). The cell lysis procedure of Saunders et al. releases chromosomal DNA, as shown by the description of multiple centrifugation and resuspension steps to remove DNA (col. 5, lines 52-56). In contrast, Applicants' claim states that the non-ionic detergent releases RNA from cells "without causing viscosity due to release of chromosomal DNA."

Second, Applicants' method mixes the biological sample with a solution containing at least about 150

mM of a soluble salt. In contrast, Sauders et al. disclose using a solution containing 75 mM NaCl and 25 mM Na_2EDTA, providing a soluble salt concentration that is less than the minimum of the claimed invention.

Because Saunders et al. do not teach every element of the claimed invention, the reference cannot anticipate the present invention. Therefore, Applicants respectfully request that the rejections under 35 U.S.C. 102(b) be withdrawn.

Rejections under 35 U.S.C. § 103

Claims 1-18 have been rejected under 35 U.S.C. §103(a), based on the combined disclosures of Eskola et al. (Clin. Biochem., 1994, 27:373-379), Kacian et al. (US Pat. 5,399,491) and Saunders et al. in view of Rowley et al. (US Pat. 5,487,970), Morris et al. (US Pat. 5,529,925), von Lindern et al. (Molec. Cell. Biol., 1992, 12:3346-3355), Goddard et al. (Science, 29 Nov 1991, pp. 1371-1374), Gruenwald et al. (US Pat. 5,858,682) and Ohki et al. (US Pat. 5,580,727).

A *prima facie* case of obviousness requires one to (1) determine the content and scope of the prior art, (2) ascertain the differences between the prior art and the claims at issue and (3) determine the level of ordinary skill in the art. *Graham v. John Deere Co.*, 383 U.S. 1, 17-18 (1966). For inventions in the biotechnology field, the level of ordinary skill in the art is generally considered to be relatively high (*e.g.*, a Ph.D. or equivalent experience). An obviousness determination also requires consideration of whether the prior art (1) would have suggested to those of ordinary skill in the art that they should make the claimed invention, and (2) would have revealed that, in so doing, those of skill in the art would have had a reasonable expectation of success. *In re Vaeck*, 947 F.2d 488, 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991).

The Examiner summarized Claims 1-18 on pages 5-7 of the Office Action. For clarification, Applicants point out that claim 2 does not refer to a "first" probe but refers to an oligonucleotide probe that binds to the first probe binding site, and Claim 15 refers to SEQ ID NOS 1 to 23, 26 and 27.

The Examiner stated that, based on the cited references, one skilled in the art at the time the invention would have been motivated to substitute the mRNA isolation method of Saunders et al. for the method used by Eskola et al., amplify via hybridization of primer sequences to an RNA population of

chromosomal translocation product RNA using the methods taught by Eskola et al. and Kacian et al., and detect via hybridization the chromosomal translocation sequences such as taught by Rowley et al., Morris et al., von Lindern et al., Goddard et al., Gruenwald et al. and Ohki et al. for rapid detection of translocation products by clinical laboratories as taught by Eskola et al. Applicants respectfully disagree, for the reasons discussed below.

Eskola et al. teach a method that requires two probes for detection: one that binds 5' of the splice junction and one that binds 3' of the splice junction (see Abstract, lines 13-16; page 374, col. 1, lines 14-16 and p. 375, col. 2, lines 26-31). Detection, as taught by Eskola et al. requires one probe that binds the amplified sequence to a solid surface (e.g., by using a biotinylated probe that binds to streptavidin coated surface) and one labeled probe that is used to detect the bound complex (see page 375, col. 2, lines 23-48). Both probes of Eskola et al. must bind for the amplified sequence to be detected. In contrast, Applicants' "hybridizing" step, uses a probe that binds to the first or the second probe binding site. Thus, Eskola et al. do not suggest Applicants' methods.

With regard to Claims 6-8, the Examiner is respectfully referred to the above discussion of Saunders et al. which distinguishes Applicants' methods from those described by Saunders et al. Because Sauders et al. teach cell lysis by using an anionic detergent followed by steps to remove released DNA, the disclosure does not suggest Applicants' methods and, in fact, teaches away from Applicants' methods.

Kacian et al. teach an amplification method that uses a promoter primer but does not teach detection of an amplified sequence that contains a splice junction as presently claimed. Kacian et al. describe, in Example 18 (col. 46-47), a system to optimize primers for use in the amplification method. In one example, use of a non-promoter primer for a t(14;18) breakpoint amplification is described, where changes in the primer site that result in a reduced number of RNase H cut sites had a positive effect on amplification efficiency (col. 47, lines 1-10). Similarly, different combinations of target sequence and promoter-primers for the major breakpoint t(14;18) translocation were also tested with regard to the effect of RNase H cut sites on amplification efficiency (col. 47, lines 40-52). This disclosure regarding primer optimization does not suggest the presently claimed methods of amplifying and detecting amplified sequences that contain a

splice junction.

Rowley et al. teach methods of detecting genetic rearrangements that rely on detection of a gene fragment or message of aberrant size or pattern (see Abstract, lines 10-17; col. 3, lines 55-60; col. 4, lines 1-7 and 32-42; col. 5, lines 4-8; col. 21, lines 43-46). The methods (e.g., Southern and Northern blotting) detect the aberrant nucleic acids using a "beakpoint-spanning" probe (col. 3, lines 17-19 and 31-33) to differentiate between normal and leukemic cells. This method does not teach Applicants' methods that do not hybridize a probe across the splice junction. By teaching detection using a breakpoint-spanning probe, Rowley et al., in fact, teach away from Applicants' methods that bind a probe to a probe binding site located either 5' or 3' of, and not overlapping, a splice junction sequence.

Morris et al. teach detection of a NPM/ALK fusion sequence by nucleic acid amplification followed by detection using two probes: one that binds to the NPM sequence and one that binds to the ALK sequence. Binding of both probes indicates the presence of the NPM/ALK fusion in the sample (col. 2, lines 1-30, particularly lines 28-30). Alternatively, Morris et al., teach using a single probe that spans the NPM/ALK fusion junction in place of the two separate probes (col. 2, lines 31-33; col. 15, lines 65-67). This is unlike Applicants' methods and does not suggest Applicants' methods that hybridizes a probe to the first or second probe binding site, neither of which overlaps the splice junction sequence.

The Examiner cited Gruenwald et al., von Lindern et al., Ohki et al. and Goddard et al. as teaching various translocations. Applicants have acknowledged that various translocations are known in the art (e.g., page 2, line 15 to page 3, line 9). Discovery or identification of a translocation is not the present invention.

Gruenwald et al. teaches detection of a chimeric protein by using monoclonal antibodies specific for the junction region or epitope of the chimeric protein (col. 2, lines 25-28, 33-34; col. 4, lines 30-36, 48-54).

von Lindern et al. describe isolation and characterization of a novel gene, *set*, that was fused to a *can* gene in leukemic cells and which produced a chimeric *set-can* transcript (page 3346, col. 2, last sentence before "MATERIALS AND METHODS"). The *set-can* fusion appears to only involve chromosome 9 genetic material because both *set* and *can* are located on chromosome 9 (p. 3351, col. 2, first paragraph).

Ohki et al. teach amplification and detection of a cDNA containing a t(8;21) fusion site by using a probe that contains the fusion site (col. 3, line 55 to col. 4, line 7) or by amplifying a sequence containing the fusion site and detecting the amplified DNA with ethidium bromide staining on a gel (col. 4, lines 19-26).

Goddard et al. describe cDNA cloning and analysis of clones containing t(15;17) translocation sequences (p. 1371, col. 1, first paragraph and col. 3, first full paragraph).

When an obviousness rejection depends on a combination of prior art teachings, there must exist some suggestion, motivation or teaching in the art to combine the references. *In re Rouffet*, 149 F.3 1350, 47 U.S.P.Q.2d 1453, 1457 (Fed. Cir., 1998). To prevent the use of hindsight in an obviousness rejection, an Examiner must show reasons that the skilled artisan, confronted with the same problems as the inventor with no knowledge of the claimed invention, would have selected the elements of the cited prior art for combination in the manner of the Applicant's invention. *Id.* at 1457-1458. The Examiner must explain the specific understanding or technological principle within the knowledge of a skilled artisan that would motivate one *without knowledge of the Applicant's disclosure* to make the combination to avoid the inference that the references were selected and combined with the assistance of hindsight. *Id.*; *In re Fine*, 837 F.2d 1071, 5 U.S.P.Q.2d 1596 (Fed. Cir. 1988)

Applicants respectfully submit that no motivation or suggestion is provided in the cited prior art to combine specific elements that are disclosed in the art cited by the Examiner and discussed above. As discussed above, a number of the cited references teach away from Applicants' invention by describing detection methods that require binding of two probes to the 5' and the 3' portions of a sequence relative to the splice junction or by using a single probe that spans the splice junction. Applicants have made clear that only a single probe that does not bind to the complementary splice junction site is needed for the present invention (e.g., page 17, lines 12-13, and page 18, lines 23-24), because the regions to be detected are so widely separated that they would not be coamplified in the absence of a splicing or translocation event (page 18, lines 20-22). This is not suggested by the cited art.

Applicants further point out that the combination of the cited references could be used to suggest a

variety of inventions that differ from the Applicants' claimed methods. For example, combining the cited prior art teachings of Saunders et al., Eskola et al.,Goddard et al. and Ohki et al. could suggest lysing cells to release RNA and DNA (Saunders et al.), amplifying the RNA or DNA using a polymerase chain reaction (Eskola et al.) using sequences specific for a t(15;17) translocation (Goddard et al.) and then the detecting the amplified sequences by examining the size of the DNA produced by staining it with ethidium bromide and visualizing it on a gel (Ohki et al.).

For the foregoing reasons, Applicants respectfully submit that a *prima facie* case of obviousness has not been established and request that the rejection of Claims 1-18 under 35 U.S.C. § 103 be withdrawn.

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CONCLUSION

In view of the foregoing amendments and remarks, the Applicants respectfully submit that the claims, as amended, are patentable and in condition for allowance. Accordingly, withdrawal of the rejections and allowance of the application is earnestly solicited. The undersigned has made a good-faith effort to address all the points raised in this Office Action and to place the claims in condition for allowance. If minor matters remain that could be resolved by telephone interview, the Examiner is invited to contact the undersigned at the number below.

Applicant believes there is no fee due in connection with the filing of this Amendment After Final. However, if Applicant is in error and a fee is required, please debit Deposit Account No. 07-0835 the appropriate amount.

Respectfully submitted,

Date: Jan. 18, 2000

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Enclosures: Exhibits 1 and 2

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